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Catalytic and structural characteristics of
2,4-diaminopentanoate dehydrogenase from *Fervidobacterium nodosum*

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In this study, I searched for thermostable 2,4-diaminopentanoate dehydrogenase (2,4-DAPDH) in order to investigate various enzymological and structural characteristics. As the result, I found it from a thermophilic anaerobic bacterium, *Fervidobacterium nodosum* Rt17-B1. In CHAPTER I, I identified the gene encoding 2,4-DAPDH and investigated various enzymological characteristics. In CHAPTER II, I synthesized various chiral amine compounds with 2,4-DAPDH. In CHAPTER III, I determined the X-ray crystal structure of the enzyme as the first experimentally determined three-dimensional structure of 2,4-DAPDH. To identify the residues involved in its catalytic function, I performed site-directed mutagenesis analysis for putative active-site residues and found that His262 and Gln264 are important for its activity. To gain a further understanding of the function of these residues, I constructed a model structure in which the cofactor and substrate are bound to the enzyme by molecular dynamics (MD) calculations.

CHAPTER I

Characterization of a thermostable 2,4-diaminopentanoate dehydrogenase from *Fervidobacterium nodosum* R17-B1

To obtain a 2,4-DAPDH with superior thermostability, I focused on the thermophilic bacterium *F. nodosum* Rt17-B1 and identified a gene, *Fnod_1646*, coding for 2,4-DAPDH. This 2,4-DAPDH is noteworthy because it has a high optimal temperature and is thermostable. The optimal temperature for this enzyme in 2,4-diaminopentanoate (2,4-DAP) oxidation was 85°C and the half-denaturation time at

90°C was 38 min. I found that besides 2,4-DAP, 2,5-diaminohexanoate can also serve as the substrate for 2,4-DAPDH. Activity of 2,4-DAPDH was suppressed by 2,4-DAP via uncompetitive substrate inhibition. In contrast, the enzyme showed typical Michaelis-Menten kinetics toward 2,5-diaminohexanoate. Enzymatic activity was decreased by the addition of various amino acids, and D-amino acids showed higher inhibitory effect than the corresponding L-amino acids in most cases. D-Tryptophan, D-phenylalanine, D-histidine, and D-leucine acted as non-competitive inhibitors, whereas D-ornithine acted as an uncompetitive inhibitor of 2,4-DAPDH. Uncompetitive substrate inhibition by 2,4-DAP and uncompetitive inhibition by D-ornithine suggest that the oxidative degradation of ornithine is regulated by upstream metabolites: at high concentrations of D-ornithine and 2,4-DAP, this degradation pathway is suppressed. The regulation of 2,4-DAPDH may also affect the metabolic fate of ornithine.

CHAPTER II

Asymmetric synthesis of chiral amine compounds with 2,4-diaminopentanoate dehydrogenase from *Fervidobacterium nodosum* Rt17-B1

Synthesis of chiral amines from prochiral ketones can be carried out with various enzymes such as transaminases and amine dehydrogenases. However, it has been difficult to synthesize chiral amines except for α-amino acids using these enzymes. 2,4-DAPDH is expected to be useful for the synthesis of amine compounds that are different from α-amino acids because it acts on the carbonyl group and amino group at the γ-position of α-amino acids. I found that 2,4-DAPDH acts on the substrates that do not have α-carboxyl group and α-amino group and catalyzes the reductive amination of 2-butanone, 2-pentanone, 2-hexanone, and acetophenone, converting them into the corresponding amine compounds. These results showed that 2,4-DAPDH has a broad substrate specificity, and α-amino group and α-carboxyl group are not absolute requirements for the substrates. I found that 2,4-DAPDH produces both *R*- and

S-enantiomers of the amine compounds. By the addition of D-tryptophan or D-histidine, which noncompetitively inhibit the oxidative deamination reaction, to the reaction mixture for the synthesis of amine compounds, I succeeded in increasing the enantiomeric purity of the amine products. In the case of phenylethylamine synthesis, D-histidine increased the e.e. value for (*R*)-1-phenylethylamine from 74% to 95%. Addition of a small molecule other than the substrate to the enzyme reaction mixture may be an effective method to increase the enantiomeric purity of the product. This method may be employed for the enzymatic synthesis of various chiral compounds.

CHAPTER III

X-ray crystallographic and mutational studies of 2,4-diaminopentanoate dehydrogenase from *Fervidobacterium nodosum* Rt17-B1

I performed X-ray crystallographic analysis of 2,4-DAPDH from *F. nodosum* Rt17-B1 to determine the three-dimensional structure of the enzyme and obtain information on the structural basis for its catalytic and regulatory properties. Site-directed mutagenesis of the enzyme was also carried out to examine the role of the amino acid residues found in the vicinity of the putative substrate-binding site. Significant loss of the activity was found for the H262A and Q264A mutant enzymes, which retained less than 1% of the wild-type enzyme activity. The H262A mutant enzyme exhibited typical Michaelis-Menten behavior, whereas substrate inhibition, which was observed for the wild-type enzyme, was observed for the Q264A mutant enzyme. These results suggested that His262 is involved not only in the expression of the catalytic activity but also in substrate inhibition observed for the wild-type enzyme, and Gln264 is involved in the expression of the catalytic activity. Furthermore, I constructed model structures for the ternary complex of 2,4-DAPDH, NAD^+, and 2,4-DAP and the quaternary complex of 2,4-DAPDH, NAD^+, and two molecules of 2,4-DAP (one of them serves as a substrate, and the other serves as an inhibitor). These

model structures provided insight into the architecture of the active site of the enzyme and the mechanism of the substrate inhibition.